PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 29 August 15, 1943 Number 8
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THE STRUCTURE AND FUNCTION OF THE GOLGI SYSTEM IN THE LIVING CELLS OF DEVELOPING MOLLUSCS

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Communicated July 14, 1943

Practically all of our knowledge of the Golgi apparatus has been built up through the study of fixed, osmic or silver, preparations, it not being generally realized that this important cytoplasmic inclusion can be revealed in many living cells by means of vital methylene blue staining. Reliance on the fixed preparations has created a great deal of confusion with regard to the structure of the Golgi bodies, because their nature is such that they only rarely lend themselves to faithful preservation by fixing fluids. In molluscs, these inclusions are of such large size that it has been possible to follow their behavior in intact embryos over periods of several days and considerable new information has been gathered regarding their structure and function.

Such observations reveal that the generalized Golgi body in molluscs is typically a more or less spherical, simple vesicle, possessing a chromophilic, gel-like, relatively lipoidal pellicle covering a relatively more protein, fluid, chromophobic core. The pellicle is thickened over one surface of the sphere forming a bowl-shaped structure which, through the microscope, looks like a crescent (Fig. 1 (*A*)). The relation between the thickened, bowl-shaped portion of the chromophile and the chromophobe is somewhat like the relation that exists between the "crescent" moon and the moon as a whole, except that in the Golgi body a thin layer of chromophilic material completely encloses the chromophobe. These generalized bodies are clearly endowed with the ability to keep the lipoidal and protein components segregated, hence the visible differentiation into chromophile and chromophobe. But they are the immediate descendants of homogeneous droplet-like forms where no such ability is manifest (Fig. 1 (*B*)). These droplets, in turn, are derived from smaller and presumably more solid granules which may be considered "reserve" forms (Fig. 1 (*C*)).

The enlargement of the granules to form droplets and the development of the droplets into larger, simple vesicles are due, with very little doubt, to the absorption of substances from the cytoplasm. This absorbing quality, together with the segregating tendency already referred to, constitute the two most fundamental physiological characteristics of the Golgi substance in these species.

In developing molluscs, the generalized Golgi bodies give rise, following gastrulation, to exceedingly active and highly specialized forms. By this period, quantities of the original yolk have been dissolved in the cytoplasm and although much has, no doubt, been oxidized, the Golgi elements

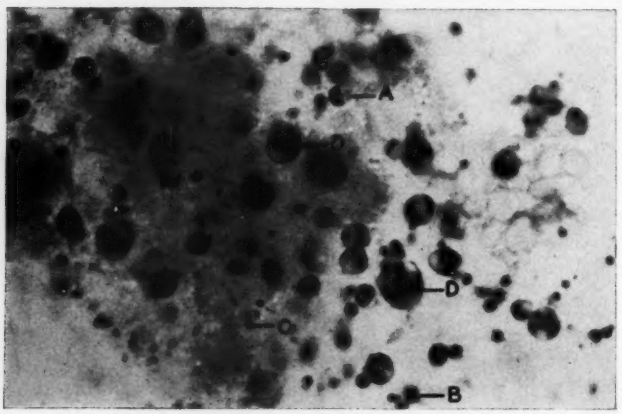

FIGURE 1

Living Golgi bodies seen in trochophore of the tectibranch Navanax (× 1000). *A*, generalized form, showing "crescent"-like chromophile, and chromophobe; *B*, droplet; *C*, granule; *D*, compound vesicular forms, each showing several chromophobes.

absorb large amounts of the material and increase considerably in size, forming elaborate, compound vesicular bodies (Fig. 1 (*D*)). Within the chromophilic material, new vesicles continually make their appearance and, in the trochophore larva, small (1μ) fat droplets are continually discharged into the cytoplasm. In the pelecypod Mytilus, the absorption by the Golgi bodies of all of the ultramicroscopic, pigmented fat particles of the single-celled egg results in the freeing of the ground cytoplasm of pigment and the deposition of this pigment in the Golgi inclusions.

The discharge from each enlarged Golgi body of large (4–6μ) protein spheres (the original chromophobes) is characteristic, in nudibranchs and tectibranchs, of the early veliger larva. In this process, the chromophilic portion of the structure withdraws from around the chromophobe and

condenses into a chromophilic, pycnotic mass, which can be shown to be rather solid. The pycnotic phase, however, is soon followed by a stage in which the mass becomes resolved into a number of spherical, relatively fluid, droplets. Each of these soon differentiates into a generalized vesicle as the segregating tendency is resumed and the cycle of the system begins anew.

The breaking up of the pycnotic Golgi body into droplets is the reproductive phase of the cycle and is the only method by means of which the Golgi elements increase in number during early development. In other words, there is no "dictyokinesis" accompanying every division of the cells, all multiplication being a mass division of the sort just described and always occurring after the assumption of a pycnotic condition following a period of marked synthetic activity. This also appears to be the situation during oögenesis where the synthetic period results in the deposition of large quantities of fat and protein yolk in the oöcyte. The multiplication period clearly increases the number of Golgi elements, thereby in tectibranchs providing a sufficient number for the new cells of the growing embryo. In the event that the embryo is not increasing in size during this period, as in Mytilus, this increase in number may be offset by a fusion phase preceding the active synthetic period. The fusion phase reduces the total number of Golgi bodies, but the original number is approximately restored during the multiplication period.

In the account just given, the internal chromophobe of each Golgi body is a potential protein or fat product which gradually becomes much more viscous as development proceeds. The elaboration of such products, in molluscs, occurs chiefly *within* the Golgi vesicle, but elaboration *outside* the vesicle is employed in Mytilus in the development of certain large oily yolk droplets. In this case, the product first makes its appearance within a group of small Golgi bodies, all of which appear to contribute to the single common yolk sphere. The mechanism involved in this process is not clear, but it is significant that elaboration of a product can take place merely through contact of the Golgi body with the ground cytoplasm.

In developing molluscs, the function of the Golgi system appears to be the continual mobilization of the fat and protein reserves within the cell. Why the embryo should continually dissolve its formed yolk inclusions, only to elaborate new ones is not immediately evident, but that the phenomenon is of fairly general occurrence in animals is indicated by the work of Schoenheimer[1] who finds that almost all of the proteins of the body are continually undergoing synthesis and breakdown. Should the Golgi system be found to be concerned in this protein turnover in higher animals, as it seems to be in the molluscs, it would prove to be a cytoplasmic constituent of greater metabolic significance than we have realized.

Summary.—For the first time, the Golgi system has been continuously followed in living, developing animal eggs. The multiplication of the Golgi elements has been observed and the rôle of the Golgi substance as an absorbing mechanism and protein and fat elaborating system is described. The active Golgi bodies are found to originate from minute, chromophilic "reserve" granules.

* Contribution from the Department of Biology, Brooklyn College, No. 45, and Contribution from The Scripps Institution of Oceanography, New Series No. 208.

[1] Schoenheimer, R., *The Dynamic Body Constituents*, Cambridge, Mass., 1942.

THE RELATION BETWEEN THE GOLGI APPARATUS AND "DROPLETS" IN THE CELL STAINABLE VITALLY WITH METHYLENE BLUE

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Communicated July 14, 1943

There has been considerable difference of opinion concerning the nature of certain "droplets" that become visible in cells when the tissue is stained with various basic vital dyes. One group of workers, notably Beams,[1] Ludford,[2] Chang[3] and others, holds that these are "neoformations" resulting from the vital dye treatment and that they have no counterpart in the unaltered, living cell. Another group of investigators, notably Covell and Scott,[4] Cowdry and Scott,[5] Ma,[6] Owens and Bensley[7] and Parat,[8] are of the opinion that these "dye droplets" represent the living Golgi material and that the classical Golgi apparatus of the fixed and osmicated cell results either from a precipitation of the osmic salt in and around these droplets, or from their running together and fusion as the result of fixation. In recent years, the tendency has swung in favor of the first contention, but crucial proof of neither of these views is at hand.

In my work, "droplets" of this kind have been revealed by means of supra- or intravital methylene blue staining of the tissues of many invertebrates, including the salivary gland cells of the larva of the midge Chironomus, and the smooth muscle, pancreas, liver and gall bladder of the frog and the smooth muscle, adipose tissue, pancreas, liver, kidney, thymus, bladder and pars distalis of the kitten. Usually, these "droplets" are opaque and show, in life, no internal structure and the reasons for their being considered "dye droplets" by so many workers are evident.

However, in all cases thus far examined, when living tissues containing

these "dye droplets" are treated with hypertonic salt solution, thus simulating the shrinkage that so frequently attends osmic acid fixation, the "droplets" rupture and form in each cell a group of fixtures that collectively so closely resemble the classical Golgi apparatus that there is little doubt but that the two are identical.

Experiments with hypertonic salt solution have shown that each "excretion droplet" is in reality a duplex structure, consisting of a rather thick,

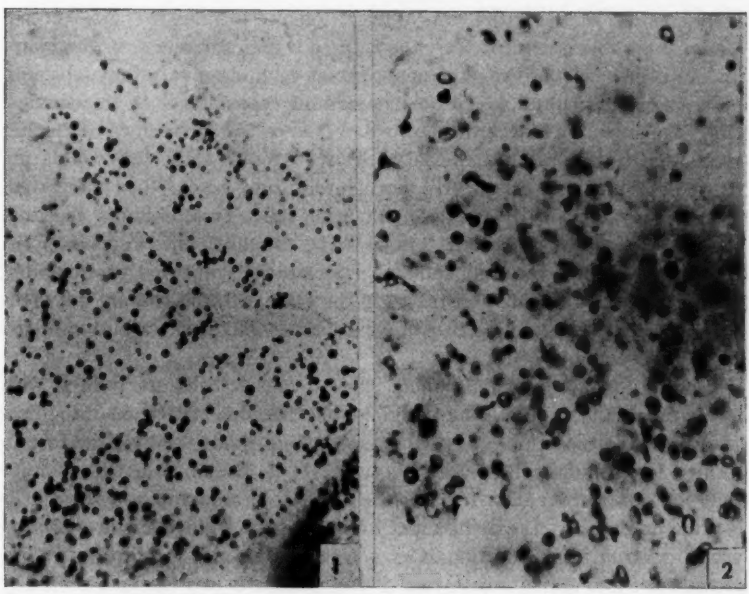

FIGURE 1

Living Golgi vesicles in the salivary gland of the larva of Chironomus, supravitally stained with methylene blue (× 500).

FIGURE 2

Same five minutes later after treatment with hypertonic salt solution (× 1000). The vesicles have ruptured, producing the classical Golgi figures. Compare with figures of Beams and Goldsmith.[9]

chromophilic, gel-like pellicle and a fluid core, the latter corresponding to the chromophobe or osmiophobe of many invertebrate Golgi bodies. The fluid interior appears to be held under some slight pressure by the pellicle and the structure, far from being a mere droplet of dye is actually a simple turgid vesicle.

When these Golgi vesicles in the relatively inactive gland cells of Chironomus (Fig. 1), for example, are treated with hypertonic salt solution.

many chromophilic rings are immediately formed, each of which usually shows a localized thickening (Fig. 2). These vesicles, therefore, have an internal structure which no ordinary dye droplet could possibly be claimed to possess. Furthermore, the pellicle is revealed by these experiments to be much thicker than would be the case of a surface membrane that might conceivably be accredited to an excretion droplet. When relatively active gland cells are similarly treated, the fluid vesicle content squirts out into the cytoplasm forming chromophilic "streamers" which immediately solidify, or the entire vesicle may elongate forming a coarse thread. Under favorable circumstances, these threads and streamers, in the mammalian acinar cells of the pancreas, contact each other forming a chromophilic pseudoreticulum which bears a striking resemblance to the classical Golgi apparatus of the osmicated cell. This resemblance may be further increased by allowing the material to desiccate on the slide, thus simulating tissue dehydration. Frequently, both intact, and ruptured or elongated, vesicles may occur side by side in the same cell. This probably accounts for the widespread belief that since "dye droplets" or "vacuome (?)" (unruptured Golgi vesicles) and Golgi apparatus (ruptured or elongated Golgi vesicles) can be demonstrated in the same cell, the two are not identical. Indeed, this may be true in some tissues, but it is certainly not the case in those mentioned above.

When vitally stained amphibian smooth muscle cells are treated with hypertonic salt solution, the Golgi vesicles become flattened into discs which, upon desiccation, due to their soft gel-like consistency often fuse to form a longitudinal plate-like structure. It is probably in this manner that the classical lamellar form of the fixed Golgi apparatus in these cells is brought about by the fixing fluid. When vitally stained tissue is treated with hypertonic salt solution after a considerable delay following its removal from the animal, distortion of the vesicles is less marked, probably because on standing the vesicles undergo a considerable increase in viscosity.

These statements are not intended to imply that true excretion droplets never occur in vitally stained tissues, but it is perfectly clear that in the tissues thus far examined, the classical Golgi apparatus of the fixed cell is formed from chromophilic vesicles of the living cell that have frequently been considered "neoformations." It appears very probable that there is a correspondence between the "vacuome" and the Golgi system in far more instances than we have been accustomed to believe. At the same time, the vital methylene blue staining technique has revealed true Golgi puddingstone-like masses in invertebrate oöcytes and developing embryos which, in section, appear network-like.

Summary.—The living Golgi system in many invertebrate and vertebrate cells consists of a series of chromophilic vesicles, each with a rather

thick gel-like pellicle enclosing a fluid core. Upon treatment of the vitally stained tissue with hypertonic salt solution, thus simulating fixation shrinkage, these vesicles can be observed to collapse, discharge the fluid vesicle content or elongate forming a pseudoreticulum similar to that exhibited by the Golgi apparatus of the osmicated cell. Desiccation, superimposed upon the shrunken material, provides an even more striking picture of the classical Golgi apparatus.

* Contribution from the Department of Biology, Brooklyn College, No. 46, and Contribution from The Scripps Institution of Oceanography, New Series No. 209.

[1] Beams, H. W., *Anat. Rec.*, **45**, 137–162 (1930).
[2] Ludford, R. J., *Proc. Roy. Soc. London*, **B107**, 101–114 (1930).
[3] Chang, H. C., *Anat. Rec.*, **62**, 95–104 (1935).
[4] Covell, W. P., and Scott, G. H., *Ibid.*, **38**, 377–400 (1928).
[5] Cowdry, E. V., and Scott, G. H., *Arch. de l'Institut Pasteur de Tunis* (1928).
[6] Ma, W., *Chinese J. Physiol.*, **4**, 381–386 (1930).
[7] Owens, H. B., and Bensley, R. R., *Am. J. Anat.*, **44**, 79–110 (1929).
[8] Parat, M., *Arch. d'Mik. Anat.*, **24**, 73–357 (1928).
[9] Beams, H. W., and Goldsmith, J. B., *J. Morph.*, **50**, 497–516 (1930).

MATTER, ELECTRICITY AND GRAVITATION IN FLAT SPACE-TIME

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Communicated July 13, 1943

In 1927 I presented two Notes[1] in which there was attempted a conceptual approach to the then new Schrödinger wave equation. This was done by taking matter to be a "perfect fluid," defined against the background of the curved space-time of Einstein's celebrated gravitational theory of 1916.

In February of last year I had the honor of presenting at Puebla and Tonantzintla, Mexico, before the Astrophysical Congress convening there, a new gravitational theory based on the same perfect fluid in the much simpler flat space-time characteristic of modern electromagnetic theory and special relativity. Already in 1912 Nordström had proposed a gravitational theory founded upon this type of space-time;[2] but his theory failed in that it did not account for the observed slight advance of the perihelion of Mercury beyond the amount indicated by the classical Newtonian theory.

My theory was obtained by laying down those demands which seemed most natural and elegant from the mathematical point of view.

In the present Note I wish to indicate in outline the modified account of matter, electricity and gravitation thus arrived at. The appropriate mathematical language is no longer that of tensors as in my two Notes of 1927, but is that of 4-vectors. It should be emphasized that, from the mathematical and philosophical point of view, the new theory is very simple.

I. Normal Coördinates in Flat Space-Time

Let ds denote the element of local time so that

$$ds^2 = dt^2 - dx^2 - dy^2 - dz^2$$

where dt and dx, dy, dz refer to the usual time and space coördinates in seconds and light-seconds, respectively. If now we replace t, x, y, z by $x^1 = t, x^2 = \sqrt{-1}x, x^3 = \sqrt{-1}y, x^4 = \sqrt{-1}z$, this formula takes the form

$$ds^2 = (dx^1)^2 + (dx^2)^2 + (dx^3)^2 + (dx^4)^2,$$

and the corresponding coördinates x^i are called normal coördinates. In such normal coördinates the language of 4-vectors becomes the same as that of 3-vectors in ordinary space. For this reason it is possible to use subscripts throughout rather than the subscripts and superscripts characteristic of tensor theory. Thus we have

$$ds^2 = dx_\alpha^2 \tag{1}$$

For brevity we shall use such normal coördinates almost exclusively.

II. The Perfect Fluid

By general consent the energy tensor of the homogeneous adiabatic fluid is written in essentially the form

$$T_{ij} = \rho u_i u_j - p\delta_{ij} \tag{2}$$

and, in terms of it, the equations of motion are written

$$\frac{\partial T_{i\alpha}}{\partial x_\alpha} = f_i. \tag{3}$$

Here ρ and $p = f(\rho)$ designate, respectively, the density and pressure of the fluid, u_i is the velocity vector dx_i/ds, δ_{ij} is the usual Kronecker δ_{ij}, and f_i stands for the body force vector per unit of volume.

The perfect fluid is singled out by the further requirement that the disturbance velocity is to be that of light at all densities, with the corresponding equation of state,

$$p = \rho/2. \tag{4}$$

Only with this type of fluid can essential mathematical difficulties be avoided at collision of portions of the fluid.[3] It is also assumed that there is an equilibrium density ρ_0 corresponding to a cosmic pressure $\rho_0/2$. The precise value of this density nowhere enters, and it would be equally possible to suppose that the equation of state has the form $p = (\rho - \rho_0)/2$ and that the pressure is 0 at the free boundaries in the customary manner.

We recall that the force vector f_i is necessarily orthogonal to the velocity vector u_i

$$f_\alpha u_\alpha = 0. \tag{5}$$

III. The General Hypotheses on the Forces

We propose now to make the following assumptions: (*a*) the force vector f_i is rational and integral in the velocity components, of not higher than the second degree; and (*b*) the coefficients are homogeneous and linear in the first partial derivatives of the corresponding potentials, namely, the atomic potential ψ which I introduced in 1927 (loc. cit.), the usual vector electromagnetic potential φ_i, and the symmetric gravitational tensor potential h_{ij} defined in the present Note. We shall furthermore suppose that (*c*) there are no degenerate quadratic terms in the velocities, i.e., no terms reducing to terms independent of u_i in view of the fundamental identity $u_\alpha^2 = 1$. These three types of potential seem appropriately designated, inasmuch as they refer primarily to matter, to electricity and to gravitation, respectively.

IV. The Atomic Potential ψ

The components of the external forces acting on unit volume of the perfect fluid due to the atomic potential will, according to our general hypothesis above, be given by a vector of the form

$$c_{i\alpha} \frac{\partial \psi}{\partial x_\alpha}$$

of degree 0 in the velocities. Since $\partial\psi/\partial x_i$ is itself a vector it follows that c_{ij} must be a (numerical) tensor, and it is immediately obvious that it can only be a multiple of δ_{ij}. In fact if u_i and v_i are any two vectors, the associated rational invariants are their squared lengths u_α^2, v_α^2 and the cosine of the angle between them, $(u_\alpha v_\alpha)/[(u_\alpha^2)(v_\alpha^2)]^{1/2}$. This fact shows that $c_{\alpha\beta} u_\alpha v_\beta$ must be $c\delta_{\alpha\beta} u_\alpha v_\beta$. Hence $c_{ij} = c\delta_{ij}$ as was stated, and the force vector under consideration must be $\partial\psi/\partial x_i$ up to a constant multiplier which may be absorbed into ψ.

But the condition (5) upon this component of f_i obviously yields

$$\frac{d\psi}{ds} = 0, \qquad (A_1)$$

i.e., *the atomic potential remains constant along the world line of any particle of the perfect fluid.* It is assumed that ψ vanishes along the free boundaries and in empty space.

The formula for the corresponding atomic body force is, of course,

$$f_{Ai} = \frac{\partial \psi}{\partial x_i}. \qquad (A_2)$$

The primordially given atomic potential ψ supplies a useful mathematical instrument in the construction of a conceptual theory of matter and electricity. Thus in 1927 (loc. cit.) I showed how the atomic potential might be used to obtain an atomic frequency equation which closely resembled the celebrated wave equation of Schrödinger.

The model atom which I proposed was not positively unstable, although without rigidity. If it be required that the elementary constituents of matter, such as the proton, electron and neutron, *cannot* become locally concave this difficulty disappears (see my Oslo paper). We would have to suppose then that, in the moment when such concavity tends to be produced, there arises a tensional normal force at the surface just sufficient to prevent it. Under such a condition a closely packed set of elementary constituents would necessarily have polyhedral forms, and this property would seem to indicate a possibility of crystalline structure and rigidity. There would obviously be a tendency of such everywhere convex bodies to maintain a roughly spherical form under collisions and other strong disturbances.

V. The Electromagnetic Potential φ_i

As indicated above, the electromagnetic force vector f_{Ei}, which arises from the terms linear in the velocities, is to be a vector of the form

$$c_{i\alpha\beta\lambda} \frac{\partial \varphi_\alpha}{\partial x_\beta} u_\lambda.$$

Since it is our intention to identify the potential φ_i with the usual electromagnetic vector potential, we impose the condition

$$\frac{\partial}{\partial x_\alpha}\left(\frac{\partial \varphi_i}{\partial x_\alpha} - \frac{\partial \varphi_\alpha}{\partial x_i}\right) = -4\pi\sigma u_i, \qquad (E_1)$$

where σ is the density of electricity. Now the only possible constituent terms in f_{Ei} are to be obtained from

$$\frac{\partial \varphi_p}{\partial x_q} u_r$$

by a single contraction of indices and choice of a subscript i, in the three possible ways:

$$\frac{\partial \varphi_i}{\partial x_\alpha} u_\alpha, \qquad \frac{\partial \varphi_\alpha}{\partial x_i} u_\alpha, \qquad \frac{\partial \varphi_\alpha}{\partial x_\alpha} u_i.$$

Consequently the force vector in question is of the form

$$a \frac{\partial \varphi_i}{\partial x_\alpha} u_\alpha + b \frac{\partial \varphi_\alpha}{\partial x_i} u_\alpha + c \frac{\partial \varphi_\alpha}{\partial x_\alpha} u_i.$$

But by the general requirement (5) this has to vanish for $i = 1$ when $u_1 = 1$, $u_2 = u_3 = u_4 = 0$, i.e.,

$$(a + b) \frac{\partial \varphi_1}{\partial x_1} + c \frac{\partial \varphi_\alpha}{\partial x_\alpha} \equiv 0.$$

Since there is no necessary relation between $\partial\varphi_1/\partial x_1$ and $\partial\varphi_\alpha/\partial x_\alpha$, we infer that $a + b = c = 0$. Hence this electromagnetic force vector is essentially

$$f_{Ei} = \sigma \left(\frac{\partial \varphi_i}{\partial x_\alpha} - \frac{\partial \varphi_\alpha}{\partial x_i} \right) u_\alpha \qquad (E_2)$$

since the ponderomotive force is proportional to the electrical density σ.

VI. The Gravitational Potential h_{ij}

By analogy with the Poisson equation in classical gravitational theory, we shall assume that a similar equation holds in each separate component of the symmetric energy tensor T_{ij} and the corresponding component h_{ij} of the symmetric gravitational potential h_{ij}, namely,

$$\frac{\partial^2 h_{ij}}{\partial x_\alpha{}^2} = 8\pi T_{ij}, \qquad (G_1)$$

where the multiplier 8π is selected for reasons of convenience. This condition (G_1) is evidently the simplest analogous equation from the formal point of view.

According to our initial hypothesis the types of terms which may enter in the corresponding gravitational force vector f_{Gi}, arising from the quadratic terms in the velocities, are derived from

$$\frac{\partial h_{pq}}{\partial x_r} u_s u_t$$

by a double contraction of indices and choice of the index i. This yields six possible types:

$$\frac{\partial h_{i\alpha}}{\partial x_\beta} u_\alpha u_\beta, \qquad \frac{\partial h_{\alpha\beta}}{\partial x_i} u_\alpha u_\beta, \qquad \frac{\partial h_{\alpha\beta}}{\partial x_\alpha} u_\beta u_i,$$

$$\frac{\partial h_{\alpha\alpha}}{\partial x_\beta} u_\beta u_i, \qquad \frac{\partial h_{i\alpha}}{\partial x_\alpha} u_\beta^2, \qquad \frac{\partial h_{\alpha\alpha}}{\partial x_i} u_\beta^2.$$

But the last two types reduce respectively to $\partial h_{i\alpha}/\partial x_\alpha$ and $\partial h_{\alpha\alpha}/\partial x_i$ since $u_\beta^2 = 1$, and so are of the degenerate type excluded by the hypothesis (c). Thus the most general available gravitational force vector is of the form

$$a \frac{\partial h_{i\alpha}}{\partial x_\beta} u_\alpha u_\beta + b \frac{\partial h_{\alpha\beta}}{\partial x_i} u_\alpha u_\beta + c \frac{\partial h_{\alpha\beta}}{\partial x_\alpha} u_\beta u_i + d \frac{\partial h_{\alpha\alpha}}{\partial x_\beta} u_\beta u_i$$

But by (5) this must vanish for $i = 1$ if $u_1 = 1, u_2 = u_3 = u_4 = 0$, so that

$$(a + b) \frac{\partial h_{11}}{\partial x_1} + c \frac{\partial h_{\alpha 1}}{\partial x_\alpha} + d \frac{\partial h_{\alpha\alpha}}{\partial x_1} = 0.$$

Since the quantities $\partial h_{11}/\partial x_1$, $\partial h_{\alpha 1}/\partial x_\alpha$ and $\partial h_{\alpha\alpha}/\partial x_1$ are independent of one another we must have $a + b = c = d = 0$. This yields for the gravitational force vector essentially the following expression

$$f_{Gi} = \rho \left(\frac{\partial h_{i\alpha}}{\partial x_\beta} - \frac{\partial h_{\alpha\beta}}{\partial x_i} \right) u_\alpha u_\beta, \qquad (G_2)$$

where the factor ρ is introduced since the gravitational force is proportional to the density ρ.

VII. The Complete Theory

We have then an energy tensor T_{ij} given by (2) with $p = \rho/2$. The equations of motion are given by (3) where

$$f_i = f_{Ai} + f_{Ei} + f_{Gi}.$$

The three terms on the right are defined by (A_2), (E_2), (G_2), and are of degrees 0, 1, 2 in the velocities. Furthermore the equations (A_1), (E_1), (G_1) determine, respectively, the atomic, electromagnetic and gravitational potentials involved. Thus we have 21 dependent variables, ρ, σ, u_i, ψ, φ_i, h_{ij}, and we have 20 equations in these variables, namely, the 4 equations of motion (3), the single equation (A_1), the 4 equations (E_1), the 10 equations (G_1) and in addition the relation $u_\alpha^2 = 1$. This is as it should be since the electromagnetic potential φ_i is only determined up to the gradient of an arbitrary function, $\partial V/\partial x_i$. It is to be remembered that in empty space we take the atomic potential ψ and the energy tensor T_{ij} to vanish.

This system of equations is complete and consistent as a mathematical embodiment of matter, electricity and gravitation. The corresponding system in the generalized theory of Einstein is incomplete to the extent that the equation of state of the homogeneous adiabatic fluid is not specified, and it is inconsistent in that when two portions of the fluid collide, the equation of motion may break down.

VIII. RÉSUMÉ OF THE NEW GRAVITATIONAL THEORY

Let us now disengage as far as possible the new gravitational theory and consider to what extent its predictions are in agreement with the known facts.

Matter is supposed to be either that special, mathematically satisfactory, homogeneous adiabatic fluid for which $p = \rho/2$ or, presumably, any form of matter in which the disturbance velocity is that of light under all circumstances.

We take T_{ij} to designate the energy tensor of matter and suppose that the equations of motion in the absence of a gravitational field (i.e., when only a small quantity of matter is present) may be written in the usual form:

$$\frac{\partial T_{i\alpha}}{\partial x_\alpha} = \bar{f}_i,$$

where $\bar{f}_i$ is a suitable force vector. It is then assumed that in the case of a gravitational field we may write the force vector f_i in the form

$$f_i = \bar{f}_i + f_{Gi},$$

where f_{Gi} designates the gravitational force vector.[4]

We assume further that there is an associated symmetric gravitational tensor potential h_{ij} such that a Poisson equation (G_1) holds in each component of h_{ij} relative to T_{ij}. In empty space T_{ij} is taken to vanish.

Under these circumstances f_{Gi} cannot be independent of the velocities since a proper force vector must be orthogonal to the velocity vector. Moreover since gravitational theory is reversible in time (in contradistinction to electromagnetic theory), and since in the classical theory the gravitational force components are given by the components of the gradient of the gravitational potential, we assume that the gravitational force vector is homogeneous and quadratic in the velocity components, and homogeneous and linear in the first derivatives of the components of the gravitational potential, and furthermore that none of its components are degenerate, i.e., involve a factor $u_\alpha^2 = 1$.

In this way we obtain for the gravitational force vector f_{Gi} the unique expression (G_2). Thus our complete system of equations is

$$\frac{\partial T_{ia}}{\partial x_a} = \bar{f}_i + f_{Gi}, \qquad \frac{\partial^2 h_{ij}}{\partial x_a{}^2} = 8\pi T_{ij},$$

where we regard f_{Gi} and T_{ij} as replaced by their explicit expressions.

It is our intention in conclusion to indicate why this simple theory of gravitation set in the framework of flat (i.e., classical electromagnetic) space-time is in agreement with the observed facts.

IX. Gravitation in the Quasi-Stationary State

Let us suppose first that the portions of the perfect fluid are moving at small velocities relative to some frame of reference so that we have approximately $u_1 = 1, u_2 = u_3 = u_4 = 0$. In this case we find in the coördinates $x^1 = t, x^2 = x, x^3 = y, x^4 = z$,

$$\Delta h_{ij} = -4\pi\rho\delta_{ij} \qquad \left(\Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}\right)$$

approximately so that h_{ij} is negligible for $i \neq j$ while h_{ii} for $i = 1, 2, 3, 4$ reduce to the ordinary gravitational potential g. The gravitational force vector per unit of mass then reduces to the gradient of g. Thus the theory is in first order agreement with the Newtonian theory.

X. The Centrally Symmetric State

Suppose next that we have a sphere of the perfect fluid at rest with its center at $(x, y, z) = (0, 0, 0)$. Of course T_{ij} and h_{ij} will then be independent of the time t, and our Poisson tensor equation reduces to the equation written above in the same coördinates so that we obtain for all i and j the exact equation outside of the sphere

$$h_{ij} = \frac{m}{r}\,\delta_{ij} \qquad (r, \text{ radial distance})$$

where m is the mass of the fluid sphere.

Thus the three *exact* equations of motion for a particle at (x, y, z) attracted by the sphere are found to be of the type

$$x'' = -\frac{mx}{r^3} - \frac{2mx}{r^3}\,(x'^2 + y'^2 + z'^2) + \frac{mx'r'}{r^2},$$

where the accent $'$ indicates differentiation as to s. It is to be observed that the first terms on the right yields the dominant Newtonian force components, the other two small terms being relativistic in origin.

Now there is no essential restriction in assuming that the initial plane of the motion through $(0, 0, 0)$ is the z-plane, whence we can at once conclude that $z = 0$ for all time. Hence we have only to solve the first two equations

with $z = z' = 0$, $r = \sqrt{x^2 + y^2}$. This is a readily integrable pair of equations with x', y' and y, $-x$ as two pairs of integrating factors.

The differential equation of the path of the particle is seen to be

$$h^2\left(\left(\frac{du}{d\theta}\right)^2 + u^2\right) = e^{2(mu + C)}(e^{2(mu + C)} - 1),$$

where $u = 1/r$ and θ is the longitude; h and C are arbitrary constants of integration. This may be integrated by an obvious quadrature.

It is thus readily established that the resultant formulas for the advance of perihelion of the particle (x, y, z) and the deviation of a ray of light (thought of as the path of a photon) in the gravitational field of the central sphere of matter are the same in their principal parts as in the Einstein theory. Furthermore the formula for the spectral shift toward the red is also in essential agreement with that theory. The exact expressions are, however, different.[5]

Thus the simple theory of gravitation here outlined seems well adapted to explain the known physical facts. Like the Einstein theory, it has the advantage of involving no arbitrary constants whatsoever. However, it is essentially different in that it presupposes a framework of flat space-time instead of space-time curved by matter, and a basic form of matter in which the disturbance velocity is that of light.

[1] "A Theory of Matter and Electricity," "The Hydrogen Atom and the Balmer Formula," these PROCEEDINGS, vol. 13, 1927. See also my article, "The Foundations of Quantum Mechanics," in the *Proceedings of the International Mathematical Congress at Oslo*, **1** (1936).

[2] "Relativitätsprinzip und Gravitation," *Physikalische Zeitschift*, **13** (1912).

[3] In a paper about to appear in the *Revista de Ciencias de Lima*, entitled "Sobre el Flúido Perfecto," I have shown by direct integration that at least in two-dimensional space-time, such difficulties do not arise with the perfect fluid.

[4] It is a mistake to believe that the Einstein theory of gravitation does not similarly *superimpose* a gravitational force upon the other forces. It is only the mechanism of the superimposition which is different in the Einstein theory. In fact any physical theory *without gravitation in flat space-time* becomes one *with gravitation in curved space-time* when ordinary derivatives are replaced systematically by the covariant derivatives of the tensor calculus.

[5] The details of the new gravitational theory will appear in my article "El concepto de tiempo y la gravitación" in the *Proceedings* of the Astrophysical Congress held at Puebla and Tonantzintla, Mexico, in February, 1942.

GROUPS OF TRANSFORMATIONS OF THE NON-INVARIANT SUBGROUPS

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Communicated June 24, 1943

All the non-invariant subgroups of a given group G are transformed under G according to a permutation group K whose degree is equal to the number of these subgroups, but not every permutation group of an arbitrary degree n is the group of transformations of the subgroups of some group. In particular, it will be proved in what follows that no imprimitive permutation group is the group of the transformations of all the non-invariant subgroups of some possible group and all the possible primitive permutation groups which are the groups of the transformations of the non-invariant subgroups of some group under this group will be determined. It therefore will remain only to determine some of the possible intransitive permutation groups which can be the groups of the transformations under a group of all its non-invariant subgroups of G.

The operators of G which correspond to the identity of K are composed of all the operators of G which transform each of its subgroups into itself and they constitute a subgroup of G which has been called the *norm* of G.[1] To emphasize the fact that they constitute an important special characteristic subgroup of G we shall call this subgroup the *character subgroup* of G. A necessary and sufficient condition that the character subgroup of G is G itself is that G is either abelian or hamiltonian. In all other cases the character subgroup of G is a proper subgroup of G whose index under G is at least as large as 2, and when it is of this index it cannot be hamiltonian and it must therefore be abelian.

To prove this fact it may first be noted that whenever the character subgroup of G is of prime index under G it involves all of the non-invariant subgroups of G. In particular, it involves all the operators of prime order contained in G. If the character subgroup of G is of index 2 under G it must therefore contain all the operators of order 2 which appear in G. There must be operators in G whose order is a power of 2 and which are not contained in the character subgroup of G. If this subgroup were non-abelian and would have an order which is a power of 2 it would also contain all the operators of order 4 which appear in G. If this were not the case an operator of order 4 which would not be found in the character subgroup of G would generate an invariant subgroup of G but its square could not be the square of the operators of order 4 found in the character subgroup of G since this subgroup contains all the operators of order 2 which appear in G.

It therefore results that the operators of order 4 which would not appear

in the character subgroup of G would have different squares. As this is clearly impossible and since not all of the operators of G which do not appear in its character subgroup could be of order 8 as a result of the properties of the possible groups of order 16 it has been proved that *whenever the character subgroup of G is of index 2 it must be an abelian subgroup of G.* When the character subgroup of G is of a larger prime index under G it is not necessarily abelian as results directly from the fact that a direct product of the quaternion group and a group which involves a prime number of conjugate subgroups, while all its other subgroups are invariant, contains a non-abelian character subgroup of prime index, where this prime is an arbitrary odd prime number.

It is known that a regular group of any prime order is the group of the transformations of all the non-invariant subgroups of some group.[2] We proceed to prove that no regular group of composite order can have this property. This will be done by showing that we arrive at a contradiction by assuming that a regular permutation group of composite order is the group of the transformations of all the non-invariant subgroups of some group. Two facts which should be kept in mind in this proof are that all the non-invariant subgroups contained in G would be conjugate under G and that all these non-invariant subgroups would appear among the operators of G which would correspond to the identity of K.

To simplify this proof we shall first consider the case when this regular group is of even order. To a permutation group of order 2 in K there would correspond in G a group whose character subgroup would be of index 2 under the group and hence this character subgroup would be abelian. Hence this group would have a commutator subgroup of order 2 and all of its operators which do not appear in the given character subgroup would generate this commutator subgroup since each of these operators would generate an invariant subgroup of G. If K would involve an operator of order 4 it may be assumed without loss of generality that this operator would generate the given permutation of order 2 contained in K. This clearly leads to a contradiction since the operators of G which correspond to the given permutation group of order 2 in K would transform all the non-invariant subgroups of G among themselves. This would also be true if K would involve a non-cyclic subgroup of order 4.

The fact to be emphasized in this connection is that the given subgroup of order 2 in K would be invariant under K since the corresponding subgroup of G would be invariant under G. If the order of K is not divisible by 4 then K must contain a permutation of odd prime order which is commutative with the given permutation of order 2. To this permutation there would correspond in G an operator which would give rise to a commutator of odd order. As this is impossible we have arrived at a contradiction by assuming that the order of K is an even number. If K would

have an odd order it is easy to see that the character subgroup of G could not have an order which is a power of 2 and hence that this subgroup would again be abelian. It therefore results that K cannot be a regular group of composite order.

If K would be an imprimitive non-regular group its subgroup composed of all its permutations which omit a given letter could not be maximal and hence G would contain non-invariant subgroups of different orders. These could not be conjugate and hence a proof of the fact that a *non-regular imprimitive group cannot be the group of the transformations of all the non-invariant subgroups of some group under this group* is a direct consequence of the fact that in such a group the permutations which omit a given letter form a non-invariant proper subgroup which is contained in a larger non-invariant proper subgroup of the imprimitive group.

When K is primitive and non-regular its subgroups composed of all its permutations which omit a given letter omit only one letter and hence the number of these subgroups in K is the same as its degree. It therefore results that these subgroups are of prime order and hence that K involves a regular subgroup as an invariant subgroup. Moreover, this subgroup must be of prime order since the number of the non-invariant subgroups of K cannot exceed its degree. It therefore results that *if a non-regular primitive group is the group of the transformations of all the non-invariant subgroups of some group under this group its order is the product of two distinct prime numbers which are such that the larger of these two prime numbers diminished by one is divisible by the smaller.* This condition is obviously both necessary and sufficient.

The intransitive permutation group of prime order and of a degree which is an arbitrary multiple of this order is the group of the transformations of all the non-invariant subgroups contained in some group. In particular, it is clearly the group of the transformations under the group of all the non-invariant subgroups of the group obtained by forming the direct product of a prime power cyclic group and groups which satisfy the condition that each contains a number of conjugate subgroups which is equal to this prime number but contains no other non-invariant subgroup. It was noted above that such groups exist for every prime number and that when the prime is odd the group itself may be the group of the transformations of its conjugate subgroups, as is the case, for instance, when the group is the symmetric group of order 6.

[1] Cf. Baer, R., *Amer. Jour. Math.*, **61**, 700 (1939).

[2] These PROCEEDINGS, **29**, 106 (1943).

ON THE UNIFORM CONVERGENCE OF THE SOLUTIONS OF THE NAVIER-STOKES EQUATIONS

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Communicated July 6, 1943

Let $\mathfrak{R}$ be a fixed region of space completely filled by a viscous fluid. We assume $\mathfrak{R}$ to be finite and to be bounded by one or more surfaces each of which may be at rest or in motion. In the case of a fluid occupying an infinite region, for example, a hypothetical infinite pipe, $\mathfrak{R}$, may be taken to be the periodicity cell which it is customary to introduce for convenience in such discussions.

Denote by $\bar{u}^\alpha$ ($\alpha = 1, 2, 3$) and $\bar{p}$ the contravariant velocity components and the pressure function of a particular (stationary or non-stationary) fluid motion in $\mathfrak{R}$. Similarly u^α and p will represent the corresponding functions for any other motion of the fluid in $\mathfrak{R}$. It will be assumed that the viscosity μ and density ρ of the fluid are constant and that all motions satisfy the Navier-Stokes equations and the equation of continuity. The quantities $\xi^\alpha = u^\alpha - \bar{u}^\alpha$ and $\xi^4 = p - \bar{p}$ are the components of an invariant ξ called the *disturbance*. We shall derive general conditions under which all such disturbances converge uniformly to zero in $\mathfrak{R}$ whereupon the above particular motion $\bar{u}^\alpha$, $\bar{p}$ will be said to be stable relative to the class of motions u^α, p (or disturbances ξ) in question.

Consider the following inequality

$$g_{\alpha\beta}\, g^{\sigma\tau}\, (\xi^\alpha_{,\sigma} + g_{\gamma\sigma}\, \xi^\alpha f^\gamma)(\xi^\beta_{,\tau} + g_{\delta\tau}\, \xi^\beta f^\delta) \geqq 0 \qquad (1)$$

valid in any Riemann space (positive definite form). In (1) the quantities $\xi^\alpha_{,\sigma}$, are the components of the covariant derivative of the spatial part of the disturbance ξ and the functions f^α are assumed to be continuous and have continuous first partial derivatives in the region $\mathfrak{R}$. Expanding the left member of (1), integrating over $\mathfrak{R}$, making use of Green's theorem and the boundary condition ($\xi^\alpha = 0$ on the boundary of $\mathfrak{R}$) we find

$$\int g_{\alpha\beta}\, g^{\sigma\tau}\, \xi^\alpha_{,\sigma}\, \xi^\beta_{,\tau}\, dV \geqq \int (f^\sigma_{,\sigma} - g_{\gamma\delta}\, f^\gamma f^\delta)\, g_{\alpha\beta}\, \xi^\alpha\, \xi^\beta\, dV. \qquad (2)$$

In the case of periodic disturbances for which $\mathfrak{R}$ is the periodicity cell the condition of spatial periodicity combined with the above boundary condition over the actual surfaces limiting $\mathfrak{R}$ suffices for the derivation of this inequality. From (2) and the energy equation for the disturbance (derived from the Navier-Stokes equations) we obtain the inequality

$$\frac{dK}{dt} + \int [\mu(f^{\sigma}_{,\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta})\, g_{\alpha\beta} + \rho D_{\alpha\beta}]\, \xi^{\alpha}\, \xi^{\beta}\, dV \leqq 0, \qquad (3)$$

in which the $D_{\alpha\beta}$ are the components of the symmetric deformation tensor of the motion $\bar{u}^{\alpha}$, $\bar{p}$ and K is the kinetic energy of the disturbance.

Now the functions f^{α} can be chosen so that the form

$$(f^{\sigma}_{,\sigma} - g_{\gamma\delta}\, f^{\gamma} f^{\delta}) g_{\alpha\beta}\, \xi^{\alpha}\, \xi^{\beta} \qquad (4)$$

is positive definite in the closure $\overline{\mathfrak{R}}$ of $\mathfrak{R}$, the condition for this being merely the existence of the condition $f^{\sigma}_{,\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta} > 0$ over $\overline{\mathfrak{R}}$. It is easily seen that this condition can be satisfied. For example if we introduce rectangular Cartesian coördinates x, y, z with origin in $\mathfrak{R}$ and take $f^1 = F(x)$, $f^2 = f^3 = 0$ the condition will be met if $dF/dx - F^2 = c^2$ where c is a constant different from zero. Integration of this equation gives $F = c \tan cx$ and hence F will be continuous and differentiable in $\overline{\mathfrak{R}}$ if c is sufficiently small. It follows that (4) can be made positive definite in $\overline{\mathfrak{R}}$ as above stated. Hence if the deformation tensor D is sufficiently small (at all times) the integrand in (3) will be positive definite. Under this condition it follows from (3) that K, the kinetic energy of the disturbance, will continually decrease and from the physical standpoint one must expect that this will result in the gradual "damping out" of the disturbance. A strict mathematical proof of the fact that the disturbance converges uniformly to zero can be made on the basis of certain assumptions concerning the continuity and finiteness of the disturbance and certain of its derivatives and application of Ascoli's theorem on the convergence of sequences. The details of proof are essentially the same as those of a proof given in a previous paper: "Qualitative Analysis of the Flow of Fluids in Pipes," *Am. Jour. of Math.*, **LXIV**, 754–767 (1942). Understanding that these conditions are met we are thus led to the following result. *Any motion $\bar{u}^{\alpha}$, $\bar{p}$ is stable in the region $\mathfrak{R}$ relative to arbitrary finite disturbances provided that its deformation tensor D is sufficiently small.* It follows in particular that if the deformation tensor vanishes the motion is always stable.

As a specific example consider the stability of Poiseuille motion in a circular pipe relative to arbitrary spatially periodic disturbances. For this motion the deformation tensor has the components $D_{13} = D_{31} = -w_0 r/a^2$ where we have referred the motion to cylindrical coördinates r, ϕ, z (the z-axis coinciding with the axis of the pipe) and where a is the radius of the pipe and w_0 the velocity of the fluid along the axis. The other components of the deformation tensor vanish. The conditions for the integrand in (3) to be positive definite can be expressed in the usual manner in terms of the elements of the symmetric matrix

$$\begin{Vmatrix} \mu(f^{\sigma}_{,\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta}) & 0 & -\frac{\rho w_0 r}{a^2} \\ 0 & \mu(f^{\sigma}_{,\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta}) & 0 \\ -\frac{\rho w_0 r}{a^2} & 0 & \mu(f^{\sigma}_{,\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta}) \end{Vmatrix}$$

On observation we see that these conditions reduce to the single inequality

$$f^{\sigma}_{,\sigma} - g_{\gamma\delta} f^{\gamma} f^{\delta} > \frac{Rr}{a^3}, \tag{5}$$

where $R = w_0 a/\nu$ is the Reynolds number, the quantity ν being the kinematical coefficient of viscosity. To treat the condition (5) let us make the substitution $f_{,\alpha} = -\psi_{,\alpha}/\psi$. The inequality (5) then becomes

$$\Delta\psi + \frac{Rr}{a^3}\psi < 0, \qquad (\psi > 0), \tag{6}$$

where the condition $\psi > 0$ is necessitated by the above requirement that the functions f_α shall be continuous and differentiable in $\mathfrak{R}$. We can suppose the inequality sign in (6) to be replaced by the equality sign as a limiting case. Or if we like we can think of this equation as involving a slightly increased value of R in which case it will be equivalent for our purpose to the strict inequality (6). With this in mind we now suppose that ψ is dependent on r alone and make the change of independent variable $x = r^{3/2}$ whereupon the condition (6) becomes

$$\frac{d^2\psi}{dr^2} + \frac{1}{x}\psi + \frac{4R}{9a^3}\psi = 0, \qquad (\psi > 0). \tag{7}$$

A solution of (7) is given by the Bessel function J_0. In fact we have

$$\psi = J_0\left(\sqrt{\frac{4R}{9a^3}}\, x\right) = J_0\left(\sqrt{\frac{4Rr^3}{9a^3}}\right).$$

The requirement $\psi > 0$ in $\mathfrak{R}$ means that the argument of the above function J_0 shall not exceed the value of the first root of this function in the interval $0 \leqq r < a$. This gives $\sqrt{4R/9} \leqq 2.4$ or $R \leqq 13$ (in round numbers).

There seem to be two distinct mathematical problems concerning the above Reynolds number R according to experimental evidence. First, it is well known that large disturbances are damped out when R does not exceed a value around 1000. However, recent experiments have shown that even for very large values of R disturbances are likewise damped out provided they are sufficiently small.[1] There appears in fact to be no upper limit to the value of R beyond which small disturbances will not converge to zero. To account for this one may have recourse to the so-called linearized equa-

tions of the disturbance which can be justified strictly only under the assumption of arbitrarily small disturbances and this theory should thus lead to the stability of Poiseuille motion relative to such disturbances for any value of the Reynolds number. In spite of this, stability has been proved only for comparatively small values of R and even then only under the additional assumption of axial symmetry. Thus Orr[2] has arrived at the value $R < 180$ for stability of Poiseuille motion relative to infinitely small axial symmetric disturbances. The problem of showing the existence of a critical value of R at approximately 1000 is a problem involving finite disturbances and the general theorem of this paper has led only to the insufficient value $R \leqq 13$ for the stability of Poiseuille motion relative to these disturbances. As far as I am aware there is no previous discussion in the literature of the stability of Poiseuille motion relative to finite disturbances on the basis of the Navier-Stokes equations.

[1] See Prandtl, L., and Tietjens, O. G., *Applied Hydro- and Aeromechanics*, McGraw-Hill, 32–35 (1934) for a more complete discussion and references to the literature.

[2] Orr, W. McF., "The Stability or Instability of the Steady Motions of a Liquid. Part II: A Viscous Liquid," *Proceedings of the Royal Irish Academy*, (A), **27**, 135 (1907). For a comprehensive discussion of stability relative to small displacements see Synge, J. L., "Hydrodynamical Stability," *Am. Math. Soc. Semicentennial Publications*, II: Addresses, 227–269 (1938).

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*RELATIONSHIP BETWEEN PARTICLE SIZE AND EFFICIENCY OF PALLADIUM-POLYVINYL ALCOHOL (Pd-PVA) CATALYSTS**

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Communicated June 21, 1943

Spectrophotometric measurements[1] carried out on PVA and silver have proved that freezing is a suitable means for obtaining reproducible changes of the size of particles of colloids. Continued investigations in this laboratory on colloidal noble metals, such as palladium, platinum and rhodium, in conjunction with synthetic high polymers and vanadium resulted in preparing supported catalysts, which have shown themselves to be extremely efficient not only from a kinetic[2] but also from a chemical[3] standpoint.

It was shown in a recent communication[4] that oriented nickel films with the same available surface as unoriented films had five times the activity as measured by ethylene hydrogenation. An attempt was made, accordingly, to obtain insight into the causes for increased activity of Pd-

PVA catalysts by coördinating ultracentrifugation of such solutions with measurements of hydrogen consumption, the rate of hydrogenation of nitrobenzene[4a] and determination of the size and shape of catalyst particles through electron micrographs.

Measurements of Gas Consumption.—First, it was attempted to determine whether any difference existed between the surface area or particle size of Pd in two types of colloidal supports. PVA was chosen as the representative member of the synthetic polymer series, and gum arabic (GA) as the representative of the naturally occurring type of colloid. The amount of gas adsorbed served as a means of measuring the surface area: for the surface area can be measured by the volume of gas adsorbed. Thus a difference in the volume of gas adsorbed by the Pd supported by PVA and by Pd supported by GA would serve as a means of distinguishing between the size of Pd particles in each medium.

Apparatus.—A thermostat was set at 30°C. ± 0.01. Special flasks were designed to fit constant pressure manometers, whereby the amounts of gas adsorbed were measured. The volume of the vessels which were cylindrical in shape was approximately 300 cc. Mercury served as the confining liquid in the manometers and dibutylphthalate was used in the supplementary capillary manometers.

Two hundred and fifty cubic centimeter samples of 1% PVA solution containing 50 mg. of Pd were prepared from 125 cc. of a 2% PVA solution, 120 cc. of distilled water and 5 cc. of a $PdCl_2$ solution containing 10 mg. of Pd per cubic centimeter, i.e., 1% with regard to Pd. As a blank, a 1% PVA solution was made up from 125 cc. of 2% PVA and 125 cc. of distilled water.

These solutions were immersed in the thermostat overnight to bring them to constant temperature. The next day, the special flasks, immersed in the thermostat and attached to the manometers, were evacuated by aspirator, filled with hydrogen and then flushed with H_2 seven times.

The hydrogen adsorption on the colloidal palladium was the difference between the PVA-Pd solution and the blank. The blank reading was added if the volume in the blank solution increased; subtracted if the volume in the blank solution decreased.

The total gas consumption figure thus obtained was diminished by the amount of hydrogen which is necessary to reduce the $PdCl_2$ (20 mg. Pd) to metallic Pd. Thus, the absolute adsorption is the total gas consumption figure minus 4.20 cc. For comparison purposes, however, the total gas consumption figure was used.

The results obtained with PVA-Pd and GA-Pd, in which the colloids were present in a concentration of 1% and 0.5%, respectively, are presented in table 1.

TABLE 1

HYDROGEN CONSUMPTION BY GA-Pd AND PVA-Pd SOLUTIONS (100 Cc.) WHICH HAVE STOOD IN THERMOSTAT 16–20 HOURS

COLLOIDAL CATALYST	CC. OF H_2 ADSORBED 0.5%	1%
GA-Pd	8.30 ± 0.20	8.30 ± 0.20
PVA-Pd	6.50 ± 0.20	3.90 ± 0.20

Two observations are of interest. First, a greater hydrogen consumption indicating a greater surface, is possessed by the inferior GA-Pd catalyst; further, an increase in consumption is shown by the PVA series when the concentration is decreased to 0.5%, whereas the GA series shows identical hydrogen consumption for both concentrations.

It had been noticed that the reddish colloidal solution containing PVA and $PdCl_2$ had turned to a blackish solution on standing for several hours (without hydrogen) in the thermostat. The GA-$PdCl_2$ solution did not exhibit this property but retained its light yellow translucent color. To circumvent this darkening, the PVA solutions were made up, let stand in the thermostat overnight and then on the next morning, immediately before introduction into the measuring vessels, made up to volume of 250 cc. by the addition of 5 cc. of the $PdCl_2$ solution. The same was done with the GA catalyst. By this treatment, no darkening occurred in the PVA solutions.

With the GA-Pd solutions, no change in hydrogen adsorption was noted. However, striking results were obtained with the PVA catalysts. PVA (1%)-Pd showed a total hydrogen consumption of 8.05 cc. ± 0.20; while a PVA (0.5%)-Pd showed a gas consumption of 8.25 cc. ± 0.20. The values given here as well as all other figures previously recorded, were the mean values of six measurements. It was apparent, then, that there was no significant difference between the adsorption of 1% and 0.5% PVA when the solutions were made up immediately before placement in the measuring vessels. These results are recorded in table 2. In table 3, all values are converted from "Total Gas Consumption" to "Absolute Adsorption," i.e., the amount of hydrogen required for reduction of $PdCl_2$ is subtracted.

TABLE 2

H_2 CONSUMPTION BY "FRESHLY PREPARED"[6] PVA-Pd AND GA-Pd CATALYSTS. 100 Cc. OF SOLUTION CONTAINING 20 MG. OF Pd ARE USED IN ALL CASES

CATALYST SOLUTION	CC. OF H_2 ADSORBED 0.5%	1%
GA-Pd	8.30 ± 0.20	8.30 ± 0.20
PVA-Pd	8.25 ± 0.20	8.05 ± 0.20

[6] "Freshly prepared" is used to designate solutions which had the $PdCl_2$ added immediately prior to the placement in measuring vessels.

TABLE 3

ABSOLUTE H_2 ADSORPTION BY "STANDING"[a] AND "FRESHLY PREPARED" PVA-Pd AND GA-Pd CATALYSTS

CATALYST SOLUTION	CC. H_2 ADSORBED 0.5%	1%
GA-Pd (standing)	4.10 ± 0.20	4.10 ± 0.20
GA-Pd (fresh)	4.10 ± 0.20	4.10 ± 0.20
PVA-Pd (standing)	2.30 ± 0.20	*0.00*
PVA-Pd (fresh)	3.85 ± 0.20	4.05 ± 0.20

[a] "Standing" is used to designate solutions wherein the PVA and $PdCl_2$ stood in contact at least 17 hours before transfer was made to the measuring vessels.

On analysis of these figures it is evident that the adsorptions observed in PVA-Pd solutions which are "freshly prepared," approximate those shown by GA-Pd. However, in "standing" PVA-Pd solutions a much smaller hydrogen adsorption than in GA-Pd was measured, and the 1% "standing" PVA-Pd showed no actual hydrogen adsorption at all, when allowance was made for the amount of hydrogen required for reduction.

The explanation of these anomalous results was that the PVA upon standing in contact with $PdCl_2$ caused a reduction of the $PdCl_2$ to metallic Pd. This would make intelligible the acquisition by the colloidal solutions on standing of a blackish shade. It appears from the data that 1% PVA is able to accomplish this stoichiometrically whereas 0.5% PVA does not fully reduce $PdCl_2$, only effecting a partial reduction in the overnight time interval.

This explained the apparent non-adsorption of hydrogen by "standing" PVA (1%)-Pd solutions, while a less active catalyst GA-Pd exhibited an adsorption of 4 cc.

Thus there was no significant difference in hydrogen adsorption displayed by PVA-Pd or GA-Pd solutions. Therefore, by this procedure no differences in particle size or available surface could be established between GA-Pd and PVA-Pd to explain the superiority of the PVA-Pd catalyst.

Additional proof for the fact that PVA reduced the $PdCl_2$ to Pd is afforded by the observation that upon determining the pH of the PVA-$PdCl_2$ solution immediately after mixing and then after standing, an increase in acidity was noted, due to the formation of HCl by the reduction of $PdCl_2$.

Ultracentrifugation and Rate of Hydrogenation.—Meanwhile, the problem of the relationship between particle size and rate of catalytic hydrogenation had been attacked from a different angle. The method of approach in this instance was to ultracentrifuge colloidal PVA-Pd solutions at various speeds, thus effecting a separation of the colloid into small Pd particles which would remain in the supernatant liquid and into the large particles which would be precipitated by the centrifuging. By quantitative deter-

mination of the Pd present in supernatant liquid and in residue and then by measuring the hydrogenation rates of equivalent quantities of Pd from both, an insight could be obtained into the effect of particle size and available surface on the activity of the catalyst. Through the courtesy of Dr. R. W. G. Wyckoff, of Lederle Laboratories at Pearl River, New York, PVA (1%)-Pd solutions were ultracentrifuged at 200 and 500 r. p. s. There was a noticeable difference in the shade of the two supernatant liquids, that which was centrifuged at the higher speed being much lighter.

The supernatant liquids were analyzed in the following way: A 1% PVA solution, which had been centrifuged at 500 r. p. s., was analyzed to determine the quantity of PVA in the top one-third and in the bottom one-third. Two cubic centimeters of the top one-third contained 18.02 mg. of total solid. The bottom one-third contained 20.50 mg. of solid per 2 cc. of solution. This gave a total solid in the two portions of 38.52 mg. per 4 cc. instead of the theoretical 40.00 mg. for a 1% solution. The total solids were then ignited for an hour to give 1.76% ash. This percentage of ash was then used to determine the quantity of Pd present in 5 cc. of supernatant liquid from the PVA-Pd solutions centrifuged at 200 and 500 r. p. s. Five cubic centimeters of supernatnat liquid were taken to dryness and weighed to give total solids. Total solids were ignited and weighed to give "ash." This "ash" was in fact ash plus Pd. The weight of ash present could be obtained by multiplying the total solids by the percentage of ash previously found, i.e., 1.76%. The weight of Pd could then be determined by substracting the weight of the ash thus obtained from the weight of Pd plus ash.

TABLE 4

DETERMINATION OF MG. OF Pd PRESENT IN 5 CC. OF SUPERNATANT LIQUID AT DIFFERENT RATES OF ULTRACENTRIFUGATION

SAMPLE	TOTAL SOLID	Pd + ASH IN MG.	% ASH IN PVA	ASH IN MG. (TOTAL SOLID × % ASH)	Pd IN MG. (ASH + Pd)—ASH
5 cc. (S. S.)[a] at 200 r. p. s.	44.43	1.73	1.76	0.78	0.95
5 cc. (S. S.) at 500 r. p. s.	43.94	1.28	1.76	0.77	0.51

[a] S. S. stands for supernatant solution.

From the data it is seen that there was twice as much Pd present in the supernatnant liquid of the sample ultracentrifuged at 200 r. p. s. as there was in the sample centrifuged at 500 r. p. s. Using these data, it was possible to set up a kinetic experiment in which the hydrogenation rate of equivalent quantities of Pd of different particle size could be compared; it is self-evident, of course, that the size of the Pd particles in the sample

centrifuged at 500 r. p. s. was smaller than that of the particles in the sample centrifuged at 200 r. p. s. If volumes of the supernatant liquids of the two centrifuged samples were taken which contained equivalent quantities of Pd, the hydrogenation rate of the supernatant liquid from the sample centrifuged at 500 r. p. s. should be greater than that of the sample centrifuged at 200 r. p. s. because of the greater surface of the former. This assumption, which correlates catalyst efficiency and particle size, proved correct.

The hydrogenation rates obtained with these two catalyst solutions containing equivalent quantities of Pd, i.e., 2 mg., are shown in figure 1, where it is demonstrated that the larger surface area in the 500 r. p. s. centrifuged sample is responsible for a greater efficiency of the catalyst.

Next the residues from the ultracentrifuging were analyzed to determine their Pd content so that aliquots containing 2 mg. of Pd could be taken and then compared with the activity of the particles of much smaller size in the supernatant liquids whose hydrogenation rates were just established and plotted in figure 1.

The results of analyses are recorded in table 5.

TABLE 5

32-CC. SAMPLE CENTRIFUGED	Pd IN 32 CC.	VOL. OF S. S.[a]	MG. Pd PER 5 CC. S. S.	TOTAL MG. Pd IN S. S.	MG. Pd IN RESIDUE
200 r. p. s.	12.48	27.0	1	5.40	7.08
500 r. p. s.	12.48	26.4	0.5	2.64	9.84

[a] "S. S." is used to designate the supernatant solution.

In order to conveniently apply the sediments, 5 cc. of distilled water were added to each bringing the volumes up to 6.3 cc. and 5.1 cc. for the 500 r. p. s. and 200 r. p. s., respectively. Thereby, 9.84 mg. of Pd and 7.08 mg. of Pd were present in 6.3 cc. and 5.1 cc., respectively. By calculation, the proportional volumes containing 2 mg. of Pd were determined and it was found that 1.28 cc. of the 500 r. p. s. sediment solution and 1.44 cc. of the 200 r. p. s. sediment solution were required. These volumes were then made up to 40 cc. in the same manner as were the supernatant liquids.

From figure 1, a convincing view of the effect of particle size on the hydrogenation rate may be obtained. For therein is presented the hydrogenation velocity toward a common substrate of analogous colloidal solutions of PVA-Pd catalysts containing equivalent quantities of Pd, viz., 2 mg. The difference between the rates of supernatant liquid and sedimented residues is striking. In the 500 r. p. s. sample, the sedimented material is about 13 times less active than the supernatant liquid using the adsorption of hydrogen at the end of five minutes as a basis for comparison: using fifteen minutes' adsorption as a basis, the sedimented material is about $7\frac{1}{2}$ times less active. With the 200 r. p. s. sample, the sediment

is about 5 times less active than the supernatant using the adsorption at the end of five minutes as a basis of comparison; using fifteen minutes' adsorption as a basis, it is about $4^1/_3$ times less active. Certainly, these ratios may only be attributed to the differences in surface area of the particles.

It was understood that the concentrations of PVA in the residue and in the supernatant solution were different, viz., 25 mg. and 90 mg., respec-

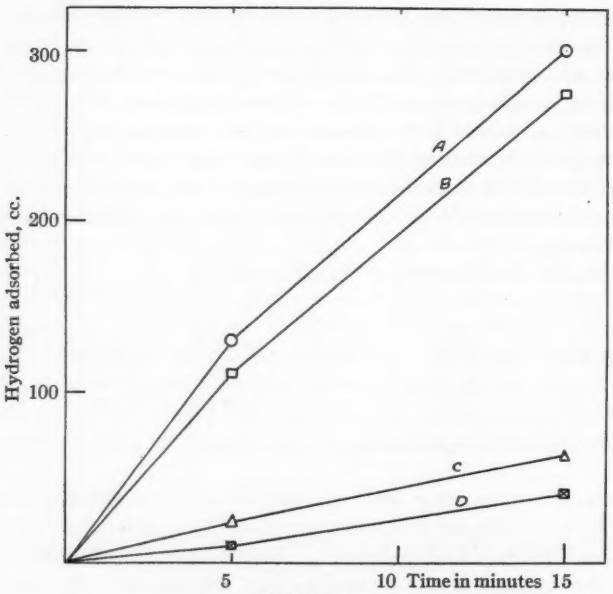

FIGURE 1

Comparison of Supernatant Liquids and Residues

Substrate: 2 mg. Pd in 40 cc. of 50% alcohol. Acceptor: 0.5 cc. $C_8H_5NO_2$. *A*, supernatant liquid of sample centrifuged at 500 r. p. s. *B*, supernatant liquid of sample centrifuged at 200 r. p. s. *C*, residue of catalyst centrifuged at 200 r. p. s. *D*, residue of catalyst centrifuged at 500 r. p. s. All measurements are converted to 25°C. and 760 mm.

tively, in the 200 r. p. s. centrifuged sample and 21 mg. and 175 mg., respectively, in the 500 r. p. s. centrifuged sample. From the data obtained by Rampino,[5] it can be seen that the effect of PVA concentration would be insufficient to explain the striking differences in the measured efficiencies of the palladium in the residue- and supernatant-catalyst.

The superiority of the supernatant liquid of the 500 r. p. s. centrifuged sample to the supernatant liquid of the 200 r. p. s. sample is also noteworthy.

FIGURE 2

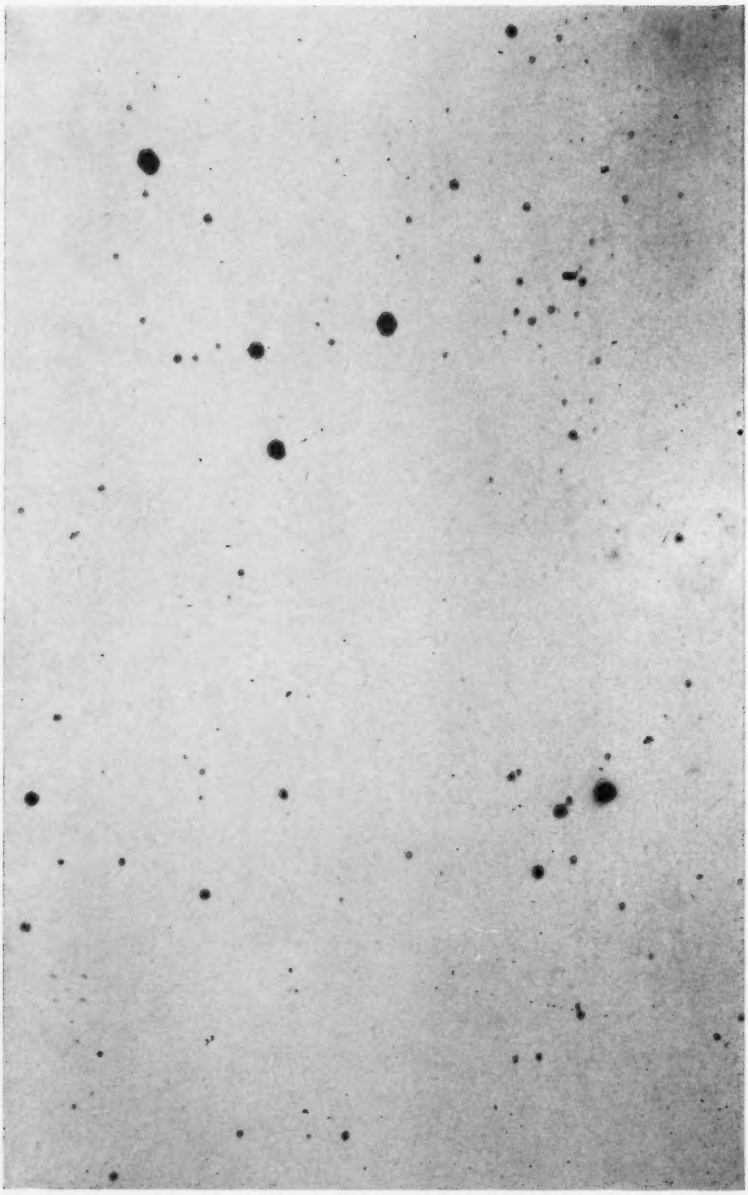

FIGURE 3

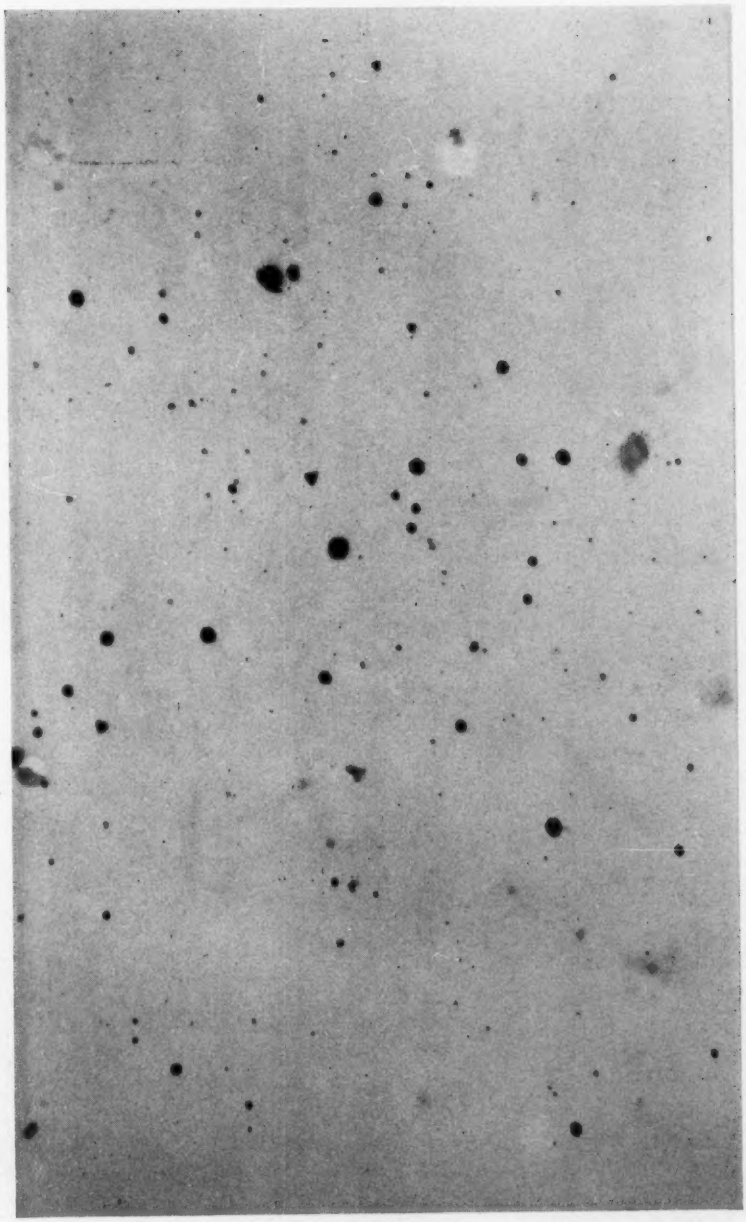

FIGURE 4

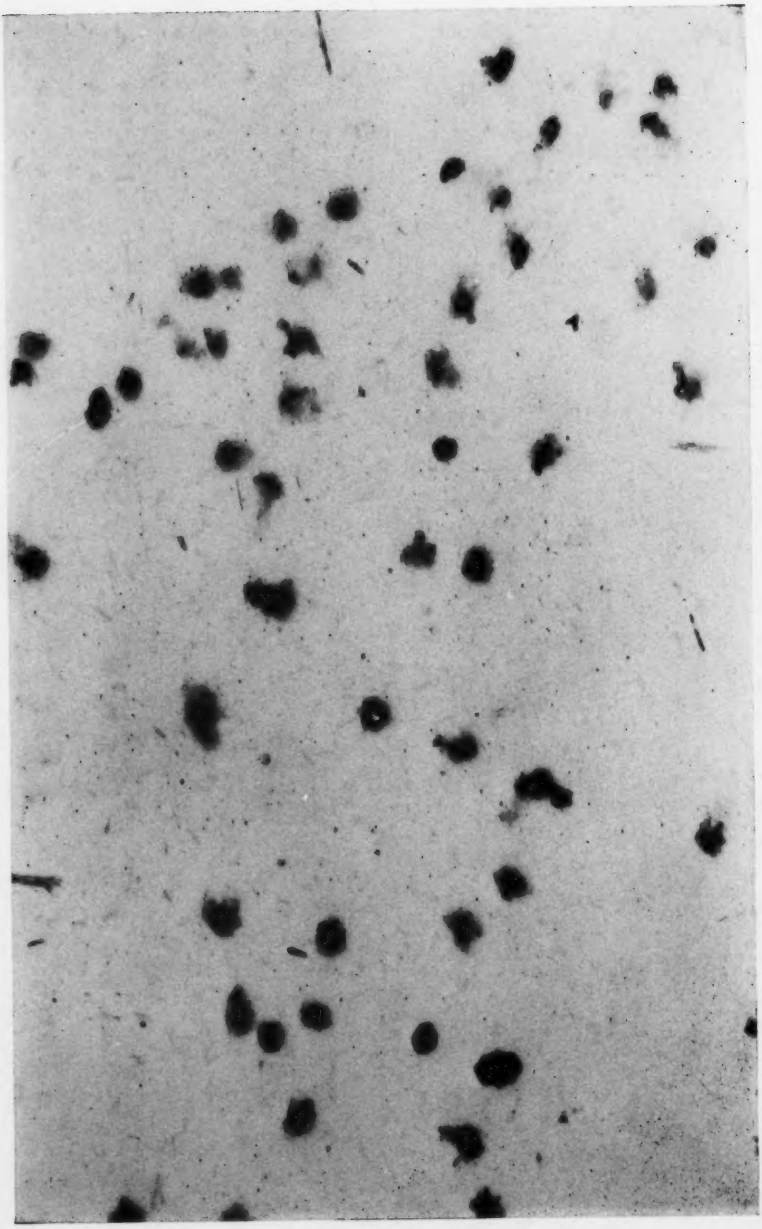

FIGURE 5

It appears from these results, that the bulk of catalytic activity rests preponderantly with the smaller particles. Thus surface area and particle size play a paramount rôle in hydrogenations performed with PVA-Pd catalysts.

Correlation of Electron Micrograph[5] Measurements and Kinetic Experiments.—Since no significant differences in particle size could be established by the gas consumption experiments previously described, it was deemed necessary to resort to a more accurate and direct means of measuring the particle size, viz., by applying the electron microscope.

Figure 2 represents an electron micrograph of PVA alone. This showed a tendency to crystallize in acicular crystals of from 0.03 to 0.4 micron in length; the shape and density of these crystals was very different from that of the Pd seen in later micrographs. This micrograph was obtained from 0.5% PVA solution magnified 30,000 times.

In figures 3 and 4 two PVA-Pd catalysts are shown. They are identical in composition: they are both 0.5% in PVA (Du Pont's-Rh-391) and contain 10 mg. of Pd per 50 cc. of H_2O. They only vary in the method used to reduce the palladium chloride to metallic Pd. The catalyst shown in figure 3 has been reduced by hydrogen, i.e., by introducing the aqueous PVA-$PdCl_2$ solution into a vessel and shaking for five minutes. The catalyst shown in figure 4 has been reduced by PVA itself, i.e., by letting the $PdCl_2$ stand in contact with the PVA whereby the slow reduction of the $PdCl_2$ to Pd, takes place. This micrograph, with its characteristic Pd particles clearly visible, conclusively proves the fact that PVA reduces $PdCl_2$ to Pd, which was proposed to explain the anomalous gas adsorption results.

In these two micrographs, the black dense metallic Pd particles are clearly visible in hexagonal crystals, in which allotropic form palladium is known to exist.[6] By far the greater number of particles assume an ultramicroscopic hexagonal configuration but some triangular shaped crystals can be noted. In these micrographs, the magnification is also 30,000 diameters. In both of these, there is no evidence of the PVA with its characteristic acicular crystals.

The possibility had been considered that these two PVA-Pd catalysts might have different hydrogenation efficiencies. The different modes of reduction could have produced Pd particles of varying sizes and of different surface areas and consequently two catalysts of varying effectiveness could have been formed. This seemed probable since one method of reduction, viz., by means of hydrogen, was of short duration, about five minutes, while the other, viz., by means of PVA, took in the case of 0.5% PVA, more than twenty hours. Kinetic experiments were run in an attempt to establish whether this difference existed. However, no significant difference could be established. Both catalysts gave hydrogenation rates of the same magnitude, within the experimental error, using nitrobenzene as an acceptor.

A statistical evaluation, wherein a size frequency curve of the single particles is determined from the electron micrographs of these two catalysts provides an explanation of their identity in kinetic behavior. A size frequency curve on the micrograph in figure 3, i.e., the PVA-Pd catalyst in which the $PdCl_2$ was reduced to Pd by hydrogen, gave a $\bar{d}_s$ size[7] (average particle size with respect to surface) of 0.07 micron. The same type of

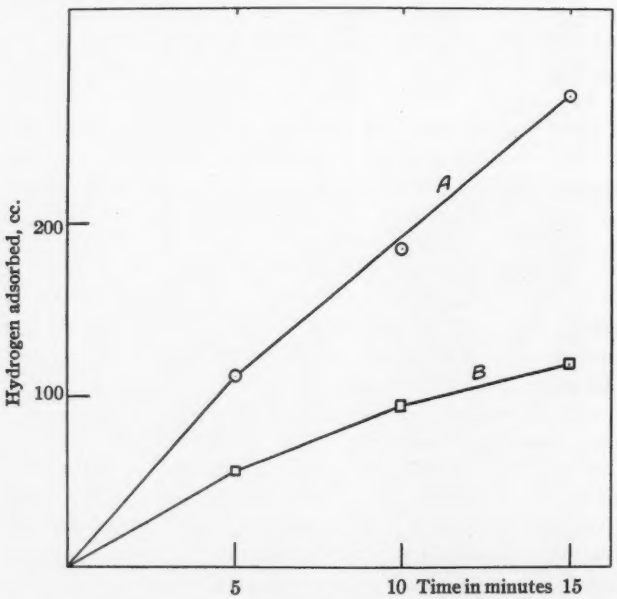

FIGURE 6

Comparison of "Supernatant" and "Normal" Catalysts
Substrate: 2 mg. Pd per 40 cc. of 50% alcohol. Acceptor: 0.5 cc. $C_8H_8NO_2$. *A*, supernatant liquid of PVA-Pd centrifuged at 200 r. p. s. *B*, normal H_2-reduced PVA-Pd. All measurements are converted to 25°C. and 760 mm.

analysis on the figure 4 micrograph, i.e., the PVA-Pd catalyst in which the $PdCl_2$ was reduced to Pd by PVA itself, gave a $\bar{d}_s$ size of 0.08 micron. There is only a slight difference in particle size of these two differently reduced catalysts, and this difference could not be expected to reveal significant deviations in the hydrogenation velocities produced by the two catalysts. Once again a relationship between surface area and catalyst efficiency is established.

Figure 5 is an electron micrograph, at a magnification of 30,000, of the

supernatant liquid of the PVA-Pd catalyst which was ultracentrifuged at 200 r. p. s. This micrograph was not made until a year and a half after the sample had been prepared and ultracentrifuged. As would be expected of a colloid which had been standing for such a length of time, a great deal of clustering had taken place, which is easily discernible in the micrograph.

However, the presence of very fine grain Pd is unmistakable. An estimate of the $\bar{d}_3$ size amounts to 0.009 micron. This value might have to be revised slightly upward because the larger particles cannot be accurately measured in the clusters. There can be no doubt, moreover, that the particle size of much of the Pd is smaller than in the previous micrographs. Using the approximation of 0.009 as the $\bar{d}_3$ size, the particle size seems to be about one-eighth that of the PVA-Pd H_2 reduced catalyst, wherein the $\bar{d}_3$ size was shown to be 0.07 micron by a size frequency curve.

It has been previously described how a kinetic experiment was run on the supernatant solution of this 200 r. p. s. sample. For comparison purposes, the hydrogenation velocity of a non-centrifuged H_2-reduced PVA-Pd catalyst, containing 2 mg. of Pd and of similar composition to the catalyst prepared from the supernatant solution of the 200 r. p. s. sample, was measured.

These two hydrogenation velocities are plotted in figure 6.

It can be seen that the "supernatant" catalyst containing the same quantity of Pd as the analogous "normal" catalyst is more than twice as efficient. The analogous "normal" catalyst had a particle size about 8 times larger than that of the "supernatant" catalyst; 0.07 micron (statistically determined) to 0.009 micron (estimated). From the results of these experiments, it is evident that there is a definite relationship between the surface area of the catalyst and rate of hydrogenation.

The only two organic colloidal catalysts to be investigated by von Ardenne[8] were Pt-Paal (sodium lysalbinate) and Pt-Skita (GA). He estimates from his micrographs that the particle size in the former is about 0.003 to 0.01 micron and in the latter, it averages about 0.025 micron. Of course, these measurements are made on platinum catalysts but it is reasonable to assume that the Pd particle size in these colloids would be of a comparable magnitude. If such an assumption is valid, certain conclusions may be drawn. The particle size of Pt and Pd, too (by analogy), in these two colloidal catalysts may be smaller, according to von Ardenne's measurements, than the particle size in PVA-Pd as determined from our micrographs; the Pt-Skita (GA) having a particle size diameter of 0.025 micron (estimated) to the 0.069 micron (calculated) diameter of PVA-Pd particles. In spite of this, the superiority of the PVA-Pd catalyst has been established. Also to be recalled is that no difference in particle size could be revealed by the gas adsorption measurements previously described. Consequently, although the preponderant effect of the particle size on the rate of hydro-

genation is definitely established, there seems to be another factor, a quality factor, the extent of which is, so far, not estimable, which is independent of particle size and which makes the synthetic high polymer carrier far superier to other colloidal carriers, e.g., GA, even though the particle size in the two types of carriers may be the same.

Whether the striking formation of ultramicroscopic hexagonal and triangular shaped Pd metal crystals in the presence of PVA has bearing on the original cause of the effectiveness of this type of catalyst, could be, perhaps, decided by comparing electron micrographs of catalysts deposited on solid bodies.

Summary.—By coördination of measurements of hydrogen adsorption, rates of hydrogenation and determination of the size and shape of catalyst particles it was established that the preponderance of catalytic activity lays with the Pd particles of smaller size. However, the superiority of the synthetic polymer supported catalyst over the GA type is to be attributed to an additional quality factor.

* This work gained the support of grants of the Bache Fund of the National Academy of Sciences, and the Penrose Fund of the American Philosophical Society, and is abridged from a part of the dissertation of K. E. K., submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy to the Graduate Faculty of Fordham University.

† Present address: Shell Development Co., Emeryville, Calif.

‡ Communication No. 30.

§ Our appreciation is expressed to Mr. Ernest F. Fullam of Interchemical Corporation of New York for his courtesy in preparing numerous electron micrographs and a discussion of the results, and to Baker and Co., Inc., Newark, N. J., for donating the noble metal salts used in this investigation.

[1] Leichter, H., and Nord, F. F., *Biochem. Z.*, **295**, 226 (1938).

[2] Rampino, Louis D., and Nord, F. F., *Jour. Amer. Chem. Soc.*, **63**, 2745, 3268 (1941).

[3] Kavanagh, Kevin E., *Ibid.*, **64**, 2721 (1942); Rampino, Louis D., and Nord, F. F., *Ibid.*, **65**, 429 (1943).

[4] Beeck, O., Smith, A. E., and Wheeler, A., *Proc. Roy. Soc.* (*London*), **A117**, 62 (1940).

[4a] Nord, F. F., *Ber.*, **52**, 1705 (1919).

[5] Dissertation, Fordham University, 1941, figure 5.

[6] Naumann, C. F., and Zirkel, F., *Elemente der Mineralogie*, 15th edition, Leipzig, Engelmann, 1907, p. 421.

[7] Green, H., *Jour. Franklin Inst.*, **204**, 713 (1927).

[8] von Ardenne, M., and Beischer, D., *Angew. Chem.*, **53**, 103 (1940).

THE DETERMINATION OF L.D.50 AND ITS SAMPLING ERROR IN BIO-ASSAY, III

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Communicated June 24, 1943

1. The discussion of this problem has been conducted on the hypothesis that the number n of animals used in each of the dilutions is the same. This restriction is convenient in that it leads to the possibility of preparing a table for the determination of the constants and of their standard errors when there are three dilutions.[1] Formulae can, however, be developed for the more general case where the numbers are not the same, but n_1, n_2, n_3. The rule of maximum likelihood leads to the equations:

$$\frac{\partial L}{\partial \gamma} = -\alpha\Sigma(2s_i - n_i) + \alpha\Sigma n_i \tanh \alpha(x_i - \gamma) = 0, \qquad (1)$$

$$\frac{\partial L}{\partial \alpha} = \Sigma(2s_i - n_i)(x_i - \gamma) - \Sigma n_i(x_i - \gamma) \tanh \alpha(x_i - \gamma) = 0. \qquad (2)$$

Placing x_1, x_2, x_3 equal to $x - c$, x, $x + c$ and setting the derivatives equal to zero, introducing $X = \tanh \alpha(x - \gamma)$ and $C = \tanh \alpha c$ we have the simultaneous equations for X and C

$$C^2[n_2X^3 - \mathbf{A}X^2 + (n_1 + n_3)X] + C(n_3 - n_1)(X^2 - 1) + \mathbf{A} - NX = 0, \qquad (3)$$

$$C^2[(n_3 - n_1)X - 2\mathbf{B}X^2] + C(n_3 + n_1)(X^2 - 1) + 2\mathbf{B} - (n_3 - n_1)X = 0, \qquad (4)$$

where

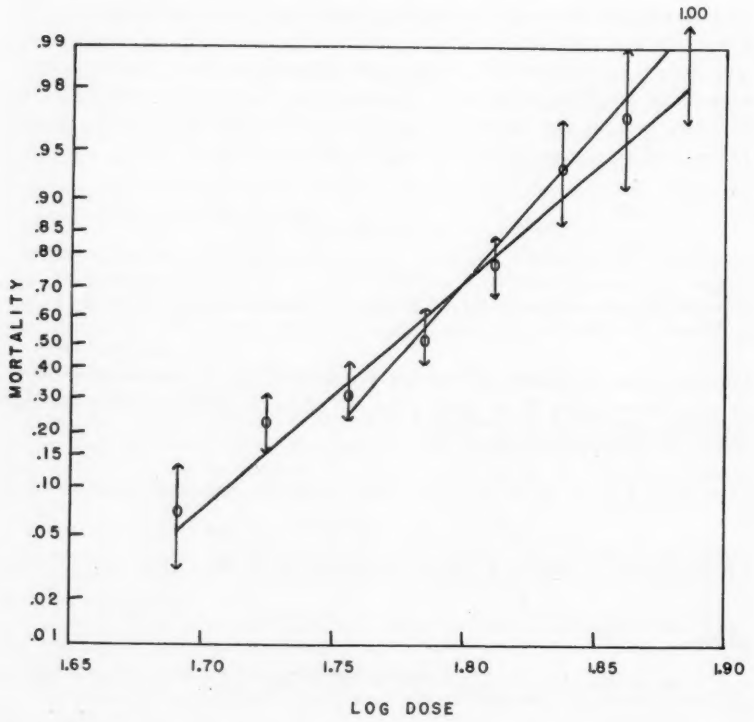
$$\mathbf{A} = 2(s_1 + s_2 + s_3) - N, \qquad 2\mathbf{B} = 2s_3 - 2s_1 - n_3 + n_1 \qquad (5)$$

and $N = n_1 + n_2 + n_3$ is the total number of animals used. Here $\mathbf{A}$ and $\mathbf{B}$ which take the place of A and B in the earlier treatment are in terms of numbers, not of proportions, of the animals affected. The elimination of C gives a cubic equation for X only slightly more complicated than that found before, viz.,

$$\begin{aligned} Nn_2X^3 - [\mathbf{A}(N + n_2) - 2\mathbf{B}(n_3 - n_1)]X^2 \\ + [\mathbf{A}^2 - 4\mathbf{B}^2 + N(n_3 + n_1) - (n_3 - n_1)^2]X \\ - [\mathbf{A}(n_3 + n_1) - 2\mathbf{B}(n_3 - n_1)] = 0. \end{aligned}$$

Having solved this numerical equation for X, one may obtain C from the equation

$$C = \frac{-\mathbf{A}(n_3 - n_1) + 2\mathbf{B}(n_3 + n_1) + n_2(n_3 - n_1)\,X}{n_2(n_3 + n_1)X^2 - [\mathbf{A}(n_3 + n_1) - 2\mathbf{B}(n_3 - n_1)]X + 4n_1n_3},$$



and then

$$\alpha = \frac{1}{c}\tanh^{-1} C, \qquad \gamma = x - \frac{1}{\alpha}\tanh^{-1} X,$$

where the functions $\tanh^{-1}$ may be replaced by their equivalents in logarithms.

The sampling errors may be obtained from

$$\frac{\partial^2 L}{\partial \gamma^2} = -\alpha^2 \Sigma n_i \operatorname{sech}^2 \alpha(x_i - \gamma) = -4\alpha^2 \Sigma n_i P_i Q_i,$$

$$\frac{\partial^2 L}{\partial \alpha^2} = -\Sigma n_i (x_i - \gamma)^2 \operatorname{sech}^2 \alpha(x_i - \gamma) = -4\Sigma n_i P_i Q_i (x_i - \gamma)^2,$$

$$\frac{\partial^2 L}{\partial \alpha \partial \gamma} = \alpha \Sigma n_i (x_i - \gamma) \operatorname{sech}^2 \alpha(x_i - \gamma) = 4\alpha \Sigma n_i P_i Q_i (x_i - \gamma),$$

when P_i are the fitted values, by

$$\sigma_\alpha^2 = -\frac{1}{H}\frac{\partial^2 L}{\partial \gamma^2}, \qquad \sigma_\gamma^2 = -\frac{1}{H}\frac{\partial^2 L}{\partial \alpha^2},$$

where H is the Hessian of L with respect to γ and α. As a matter of fact these formulae are valid for any number of dilutions provided a solution for α and γ and consequently for P_i upon the curve has been found in any manner, presumably by successive approximation from the equations

$$\left.\frac{\partial L}{\partial \gamma}\right|_0 + \left.\frac{\partial^2 L}{\partial \gamma^2}\right|_0 \delta\gamma + \left.\frac{\partial^2 L}{\partial \alpha \partial \gamma}\right|_0 \delta\alpha = 0, \qquad \left.\frac{\partial L}{\partial \alpha}\right|_0 + \left.\frac{\partial^2 L}{\partial \alpha \partial \gamma}\right|_0 \delta\gamma + \left.\frac{\partial^2 L}{\partial \alpha^2}\right|_0 \delta\alpha = 0.$$

2. In case one is working with a sufficient number of dilutions and in case the observed values P_i do not fluctuate too much from a straight line on growth paper one may get an approximate solution by graphical methods. For example, if we have the following logarithmic doses x and proportions of population affected[2]

x	1.755	1.784	1.811	1.837	1.861	1.884
n	28	27	30	31	30	29
s	9	14	23	29	29	29
P	0.329	0.519	0.767	0.936	0.967	1.000

we may plot the first 5 points ($P = 0$ or 1.0 cannot be plotted) and draw the line. The 50% end-point is read as at 1.777. The fitted growth curve being

$$P_f = {}^1/_2 + {}^1/_2 \tanh \alpha\,(x - 1.777), \qquad \alpha = \frac{\tanh^{-1}(2P-1)}{x - 1.777}$$

permits the determination of α from any values P_f and x read from the line. At $x = 1.837$, $P_f = 0.935$. Hence $\alpha = 22.2$ and $P_f = {}^1/_2 + {}^1/_2 \tanh 22.2\,(x - 1.777)$ is the line as determined graphically.

3. The values of P_f as fitted may be read[3] from the line and the calculation form may be filled out.

x	1.755	1.784	1.811	1.837	1.861	1.884
P_f	0.280	0.587	0.810	0.935	0.975	0.992
Q_f	0.720	0.413	0.190	0.065	0.025	0.008
$P_f - P$	−0.049	0.068	0.043	−0.001	0.008	−0.008
$s_f - s$	−1.37	1.84	1.29	−0.03	0.24	−0.23
$x - \gamma$	−0.022	0.007	0.034	0.060	0.084	0.107
$(s_f - s)(x - \gamma)$	0.0301	0.0129	0.0439	−0.0018	0.0202	−0.0246
$4nP_fQ_f$	22.6	26.1	18.5	7.5	2.9	0.9
$4nP_fQ_f(x - \gamma)$	−0.50	0.18	0.63	0.45	0.25	0.10
$4nP_fQ_f(x - \gamma)^2$	0.011	0.001	0.021	0.027	0.021	0.011

Now,

$$\left.\frac{\partial L}{\partial \gamma}\right|_0 = 2\alpha\Sigma(s_f - s) = 77.3, \qquad \left.\frac{\partial L}{\partial \alpha}\right|_0 = -2\Sigma(s_f - s)(x - \gamma) = -0.161,$$

$$\left.\frac{\partial^2 L}{\partial \gamma^2}\right|_0 = -38{,}700, \qquad \left.\frac{\partial^2 L}{\partial \alpha^2}\right|_0 = -0.092, \qquad \left.\frac{\partial^2 L}{\partial \alpha \partial \gamma}\right| = 24.6.$$

Then $77.3 - 38{,}700\delta\gamma + 24.6\delta\alpha = 0$, $-0.161 + 24.6\delta\gamma - 0.092\delta\alpha = 0$ give as solutions $\delta\gamma = 0.00106$, $\delta\alpha = -1.47$ and $\gamma = 1.77806$, $\alpha = 20.73$ as a first arithmetic approximation subsequent to the graphical fit. If the graphical fit be considered an adequate approximation for computing σ_γ and σ_α we have for the Hessian $H = 2955$ and hence

$$\sigma_\alpha^2 = \frac{38{,}700}{2955} = 13.1, \qquad \sigma_\gamma^2 = \frac{0.092}{2955} = 0.000031,$$

which gives $\sigma_\alpha = 3.6$, $\sigma_\gamma = 0.0056$ and we write[4]

$$\gamma = 1.7781 \pm 0.0056, \qquad \alpha = 20.7 \pm 3.6.$$

In this example the logarithmic doses x did not advance by uniform amounts so that it is impossible satisfactorily to determine α and γ from the table which was computed on the basis of an equal advance in x and constant values of n; but if one should ignore these points and take the 1st, 3rd and 5th observed P's we should find $A = 1.126$, $B = 0.638$ giving "γ" $= -0.63$ and "α" $= 1.00$. With $c = 0.053$ we have $\gamma = -0.033$ and $\alpha = 19$. The 50% end-point would be at $1.811 - 0.033 = 1.778$. The values 1.778 and 19 in place of 1.777 and 22.2 determined graphically or 1.7781 and 20.7 obtained by the first arithmetic approximation are much the same thing in view of the standard errors estimated from the graphical solution. We could not expect to get the values of the standard deviations from the table because they would be estimated from only about one-half of the observations and furthermore the estimates depend on the actual points used on the growth curve as well as on the number of observations; in fact a calculation gives 1.778 ± 0.0080 and 19 ± 4.2 in place of 1.7781

$\pm$ 0.0056 and 20.7 $\pm$ 3.6. In this case, however, the use of the table and only three of the six observed proportions gives a fairly good answer.

4. There need be no restriction to three points or to identical values of n_i. If in (1) and (2) we write $x_i = x + ic$, assuming the logarithmic doses to proceed in a uniform manner (or the dilutions to be a geometric progression) we have for the determination of γ and α

$$-\alpha\Sigma(2s_i - n_i) + \alpha\Sigma n_i \tanh \alpha(x + ic - \gamma) = 0,$$
$$\Sigma(2s_i - n_i)(x + ic - \gamma) - \Sigma n_i(x + ic - \gamma) \tanh \alpha (x + ic - \gamma) = 0.$$

If the first equation be multiplied by $(x - \gamma)/\alpha$ and added to the second, the latter becomes

$$\Sigma i(2s_i - n_i) - \Sigma i n_i \tanh \alpha (x + ic - \gamma) = 0.$$

The equations will be algebraic in $X = \tanh \alpha(x - \gamma)$ and $C = \tanh c$ but of too high an order to be readily used. It will, however, be noticed that the solution depends on the particular two constants

$$\mathbf{A} = \Sigma(2s_i - n_i), \qquad 2\mathbf{B} = \Sigma i\,(2s_i - n_i).$$

For this reason, if some standard set-up involving more than three dilutions in geometric progression were to be adopted for wide-spread use it would be possible, and might be well, to compute a double entry table for[5] γ and α and their standard deviations in terms of **A** and **B**.

[1] These PROCEEDINGS, **29**, 79–85, 114–120, 150–154, 207–212 (1943).

[2] For growth paper, see Wilson, E. B., these PROCEEDINGS, **11**, 451–456 (1925). The data used in the illustration are taken from Bliss, C. I., p. 154 of *Ann. Applied Biol.*, **22**, 134–167 (1935) or from Irwin, J. O., p. 17 of *Suppl. Jour. Roy. Stat. Soc.*, **4**, 1–60 (1937). Those authors fit only the last six of the eight points. In the figure the limits of P at one standard deviation as determined from the formula on p. 209 of *Jour. Amer. Statis. Assoc.*, **22**, 209–212 (1927) have been inserted and two lines have been drawn, one fitting (graphically) the last six and the other all eight points. It is clear that the graphical fit to the eight points is not bad; indeed if one actually fits the eight points arithmetically one finds $\gamma = 1.7714 \pm 0.0056$, $\alpha = 17.1 \pm 2.1$ with a χ^2 value of 3.35 which for six degrees of freedom gives $P = 0.76$ with a probability that because of the small numbers in some of the cells, the true value of P would be even larger. It is true that the deviations seem to be of such signs as to indicate curvature of the observed points to the fitted line, but in view of the small value of χ^2 and high value of P one might hesitate to draw that inference and to reject the first two points from this statistical evidence. The real reason for the rejection of these two points lies in the fact that in other corresponding sets of data the mortalities at low dosages seem also to be too high to fit the growth curve or probability integral curve. However, if the true form of the curve fitting such data were known, the values of the 50% end-point and of its standard error might be somewhat different from those found by fitting either of these curves to the last six points.

[3] It might be better to calculate the values of P_f from the analytic expression even for the first arithmetic approximation to improve the value graphically determined, and calculation would certainly be necessary for a second arithmetic approximation.

[4] If the arithmetic approximations were continued we should find $\gamma = 1.7787$, $\alpha = 20.53$. The value of χ^2 would be such as to give $P = 0.92$ for the fit to the six points, which would indicate that the variance of the observed from the fitted points was only 0.29 of the amount which on the average would be due to chance or that the variation of the observed from the fitted points would be only 0.54 of the chance amount.

[5] For the actual computation of such a table one would not go back to the general and approximative equations which one would use to determine α and γ for given values of **A** and **B**. As the values of **A** and **B** are the same for the observated data and for the values fitted to them, one would start with assumed values of "γ" and "α" and determine **A** and **B** from the equations.

$$\mathbf{A} = \Sigma n_i \tanh \text{“}\alpha\text{”} (i - \text{“}\gamma\text{”}), \qquad 2\mathbf{B} = \Sigma i n_i \tanh \text{“}\alpha\text{”} (i - \text{“}\gamma\text{”}).$$

This would give a table for **A** and **B** in terms of "γ" and "α" from which the table for "γ" and "α" could be obtained by inversion.